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Changes of Plasma Hemostatic Markers during Percutaneous Transluminal Coronary Angioplasty in Patients with Chronic Coronary Artery Disease

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Changes of hemostatic parameters during percutaneous transluminal coronary angioplasty (PTCA) in 75 patients with chronic coronary artery disease were evaluated. Plasma levels of D-dimer, soluble fibrin monomer, plasmin-α2 antiplasmin inhibitor complex, and tissue factor (TF) were significantly increased in all patients with chronic coronary artery disease. The activity of antithrombin and protein C and the levels of protein C antigen were significantly decreased 1 hr after PTCA, but they returned to normal range 1 day after PTCA. There was no significant difference in the level of plasma APC-PCI complex before and 1 hr after PTCA. The plasma levels of D-dimer, soluble fibrin monomer, thrombomodulin, TF and PPIC were significantly decreased 1 hr, and the plasma levels of plasmin-α2 antiplasmin inhibitor complex 1 day after PTCA. These findings suggest that the decrease of protein C and antithrombin resulted in activation of the coagulation system. One hour after PTCA, the plasma levels of (total-free) TF pathway inhibitor (TFPI) were significantly decreased, but the plasma levels of total and free-TFPI were significantly increased, suggesting that comsumption of (total-free) TFPI occurs during PTCA. Overall, these findings suggest that the hypercoagulable state improves during PTCA and that transient decrease of antithrombin, protein C, (total-free) TFPI or plasmin-α2 antiplasmin inhibitor complex may cause restenosis of coronary artery. Am. J. Hematol. 61:238–242, 1999. © 1999 Wiley-Liss, Inc.

Key words: PTCA; chronic coronary artery disease; protein C; TF; TFPI

INTRODUCTION

Although acute myocardial infarction (AMI) may be fatal, survival, and cardiovascular morbidity improve by thrombolytic therapy or by percutaneous transluminal coronary angioplasty (PTCA) [1]. It was reported that the re-occlusion after PTCA can be prevented by high dose of heparin [2]. Hypercoagulability is associated with ischemic heart disease [3,4], and it has been particularly involved in the pathogenesis of AMI, and in the re-occlusion of coronary artery after PTCA. Coronary thrombosis generally occurs at sites of stenosis, which is oftentimes precipitated by fissuring of an atherosclerotic plaque [5]. Because atherosclerotic plaques contain tissue factor (TF) synthesizing cells, their rupture induces activation of the TF pathway [6], leading to thrombin formation, platelet activation and fibrin deposition. Elevated plasma tissue factor pathway inhibitor (TFPI) activity and Factor VII have been demonstrated in patients with AMI [7,8]. TFPI was initially identified after the observation that preincubation of TF with serum prevented the lethal effect of disseminated intravascular coagulation that occurs after TF infusion in animals [9,10]. TFPI directly inhibits F Xa and also the proteolytic activity of F VIIa/TF complex by forming a quaternary

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TABLE I. Hemostatic Data in Patients with Coronary Artery Disease before PTCA

	Patients	Healthy Volunteers
APTT (s)	31.8 ± 18.6	31.5 ± 2.3
PT (s)	16.2 ± 9.17	11.9 ± 0.75
Antithrombin (%)	87.7 ± 13.1	97.6 ± 9.5
Protein C activity (%)	99.4 ± 19.5	104.5 ± 10.8
Protein C antigen (%)	112.0 ± 40.8	106.2 ± 19.4
D-dimer (ng/ml)	759 ± 837*	258 ± 173
SFM (μg/ml)	11.6 ± 29.3*	2.41 ± 2.95
PAP (μg/ml)	0.89 ± 0.53*	0.44 ± 0.18
TM (ng/ml)	11.8 ± 8.1	10.5 ± 2.9
TF (pg/ml)	177.1 ± 87.6	143.5 ± 34.3
Total-TFPI (ng/ml)	72.9 ± 39.3	68.7 ± 14.1
Free-TFPI (ng/ml)	24.7 ± 27.3	17.7 ± 5.4
(total-free) TFPI (ng/ml)	46.9 ± 19.4	51.1 ± 12.0

*$P < 0.05$.

TABLE II. Changes of Hemostatic Data in Patients with Coronary Artery Disease during PTCA

	Before	After 1 Hr	After 1 Day
APTT (s)	31.8 ± 18.6	prolonged#	34.0 ± 23.8
PT (INR)	16.2 ± 9.17	41.8 ± 20.3**	16.7 ± 9.56
Antithrombin (%)	87.7 ± 13.1	73.6 ± 12.6**	84.9 ± 12.6
Protein C activity (%)	99.4 ± 19.5	72.0 ± 14.4**	92.2 ± 16.7
Protein C antigen (%)	112.0 ± 40.8	99.9 ± 33.6*	100.4 ± 38.7
D-dimer (ng/ml)	759 ± 837	481 ± 525*	560 ± 643
SFM (μg/ml)	11.65 ± 29.28	8.28 ± 22.92	7.15 ± 25.51
PAP (μg/ml)	0.89 ± 0.53	0.74 ± 0.41*	0.57 ± 0.25**
TM (ng/ml)	11.77 ± 8.08	9.48 ± 3.02*	12.83 ± 6.89
TF (pg/ml)	177.1 ± 87.6	152.3 ± 59.1*	164.6 ± 50.7
Total-TFPI (ng/ml)	72.9 ± 39.3	177.5 ± 67.6**	69.2 ± 36.3
Free-TFPI (ng/ml)	24.7 ± 27.3	139.3 ± 68.5**	23.9 ± 29.7
(total-free) TFPI (ng/ml)	46.9 ± 19.4	37.2 ± 22.2**	42.9 ± 17.2

*$P < 0.05$, **$P < 0.01$.
#APTT could not be measured because it was markedly prolonged.

complex (F Xa/TFPI/F VIIa/TF). TFPI is synthesized mainly by endothelial cells, and exists on the endothelium and in plasma in vivo [11,12]. In plasma, TFPI is present in a free form or associated with lipoproteins. The level of plasma TFPI and the proportion of each form of TFPI vary in several pathophysiological conditions [13–16]. In the present study, we measured the plasma levels of hemostatic factors during PTCA in patients with chronic coronary heart disease.

MATERIALS AND METHODS

Seventy-five patients (male, 65; female, 10; mean age, 64.6 ± 9.2 years old) that underwent elective and uncomplicated PTCA and 30 healthy volunteers (male, 20; female 10) were enrolled in this study. Patients with acute coronary syndrome, malignant tumors, infections, collagen vascular disease and those under oral anticoagulant therapy or heparin treatment were excluded from the investigation. The site of coronary artery disease was the right coronary artery in 25 patients, the circumflex branch in 19 patients, the anterior descending coronary artery in 28 patients and the left intrathoracic artery graft in 3 patients. There were 30 cases of stable angina and 45 patients with old myocardial infarction.

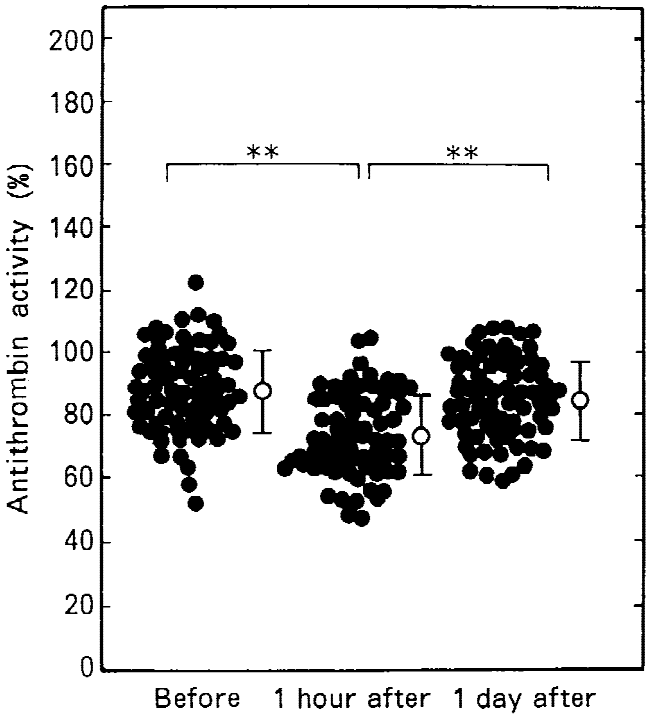

Fig. 1. Plasma activity of antithrombin in patients with chronic coronary artery disease during PTCA. **$P < 0.01$.

PTCA was performed by experienced cardiologists according to standard methods and it was considered successful if the minimal lumen diameter increased more than 20% of baseline, and if the residual stenosis was less than 50%. A bolus of heparin (10,000 IU) was injected intravenously in all cases immediately before PTCA; no additional heparin administration was done during or after PTCA. In addition to nitrates, β-blockers, or calcium channel blockers alone or in combination, all patients received acetylsalicylic acid (81 mg/day) and ticlopidine hydrochloride (200 mg/day). Data obtained in 30 healthy volunteers served as controls. Informed consent was obtained from each subject before PTCA.

Blood sampling was done before, 1 hr and 24 hr after PTCA. For the assays, whole blood was anticoagulated by the addition of 9 volumes of blood to 1 volume of 3.8% trisodium citrate solution. Activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen activity were measured by clotting method by using a Patromtin (Behringwerke AG, Marburg, Germany), Thromborel S (Behringwerke AG), and Multifibren U (Behringwerke AG), respectively. The activity of plasma antithrombin and protein C (PC) was measured

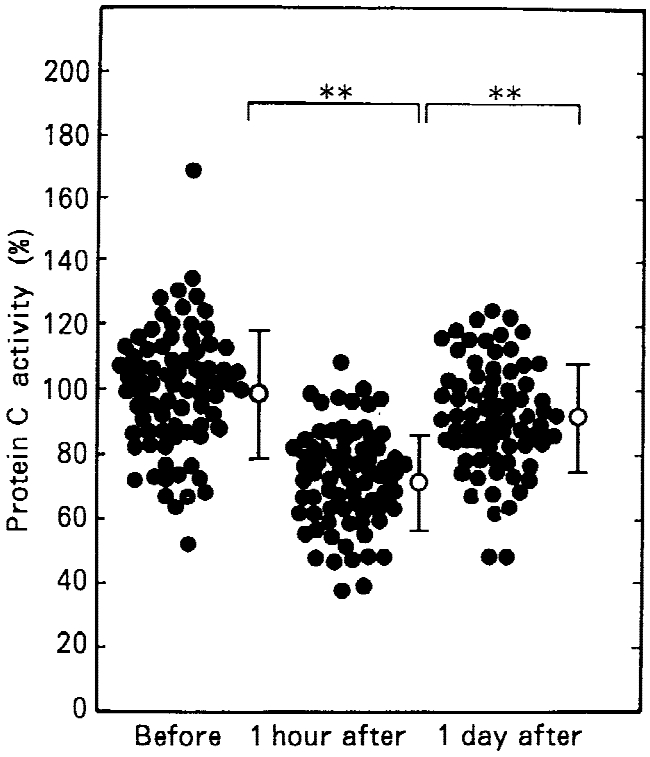

Fig. 2. Plasma activity of protein C in patients with chronic coronary artery disease during PTCA. **$P < 0.01$.

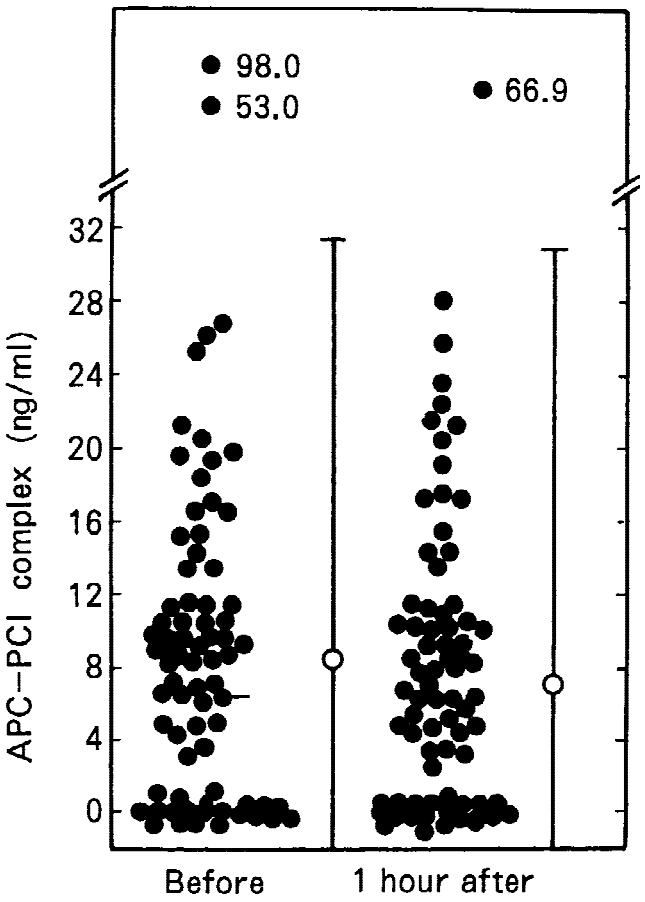

Fig. 3. Plasma level of APC-PCI complex in patients with chronic coronary artery disease during PTCA.

by amidolytic assays, by using Berichrom AT III and Berichrom-Protein C (Behringwerke AG). Plasma antigen levels of PC was measured by enzyme-linked immunosorbent assay (ELISA), by using anti-PC and antibodies (Dakopatts, Glostrup, Denmark). The plasma levels of plasmin-α2 antiplasmin complex (PAP), FDP-D-dimer, and thrombomodulin (TM) were determined with PIC-test-Teijin-F (Teijin), D-dimer test-Teijin-F (Teijin, Tokyo, Japan) and TM-test-Teijin (Teijin), respectively. Plasma activated protein C (APC) protein C inhibitor (PCI) complex levels were measured by sandwich ELISA [17].

Free-TFPI, total-TFPI, and TF antigen levels were measured by ELISA kits from Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan), based on the one-step sandwich ELISA method. Free-TFPI was measured by using two different monoclonal antibodies against TFPI obtained by cell fusion method [18,19]. The monoclonal antibody immobilized on microplate wells recognized the K3 domain [16], and the monoclonal antibody conjugated to horse radish peroxidase (HRP) recognized the specific conformation formed between the K1 and K2 domains [19]. Total-TFPI antigen level was measured by using polyclonal and monoclonal antibodies [20]. The rabbit anti-TFPI polyclonal antibody was immobilized on microplate wells, and the monoclonal antibody, which recognizes the specific conformation formed between the K1 and K2 domains, was conjugated to horse radish peroxidase (HRP).

Data was expressed as mean ± standard deviation. Statistical difference between two groups was assessed by the Wilcoxon's rank test and that among three groups by analysis of variance.

RESULTS

The diameter of the stenotic coronary artery was 89.3 ± 8.3% before PTCA, and it was reduced to 18.8 ± 18.1% after PTCA. Although APTT was within normal range in patients with coronary artery disease, PT was slightly prolonged. The activity of plasma antithrombin and protein C was almost normal in all patients, but the plasma levels of D-dimer, SFM, and *PAP* were significantly increased ($P < 0.05$, respectively). The plasma level of TF was increased ($P < 0.05$), but the plasma levels of total-TFPI, free-TFPI, and (total-free) TFPI were within normal range (Table 1). Changes of hemostatic factors in all patients during PTCA are described in Table 2. APTT and PT were significantly prolonged 1 hr after PTCA ($P < 0.01$), but they returned to normal range 1 day after

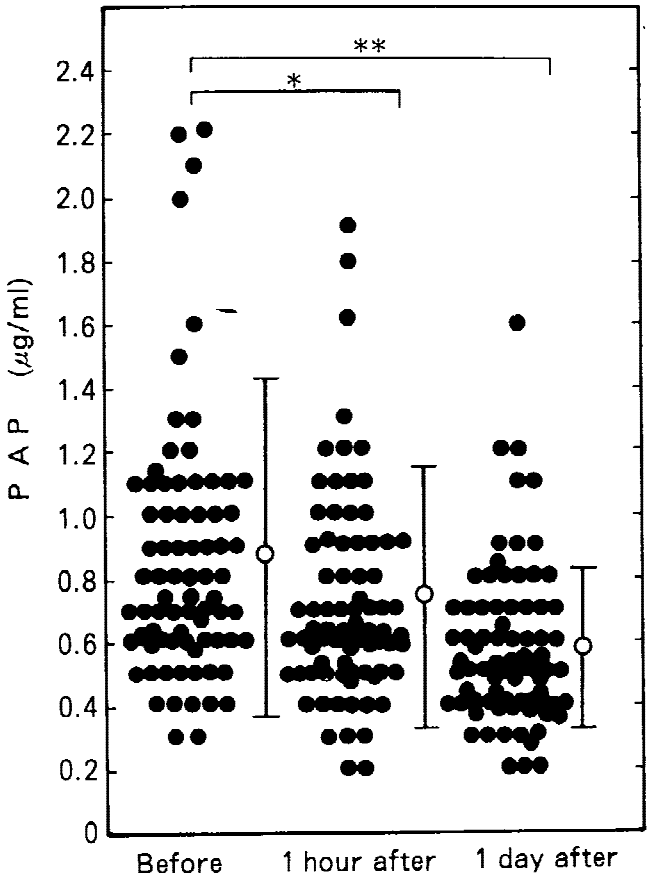

Fig. 4. Plasma level of *PAP* in patients with chronic coronary artery disease during PTCA. **P* < 0.05, ***P* < 0.01.

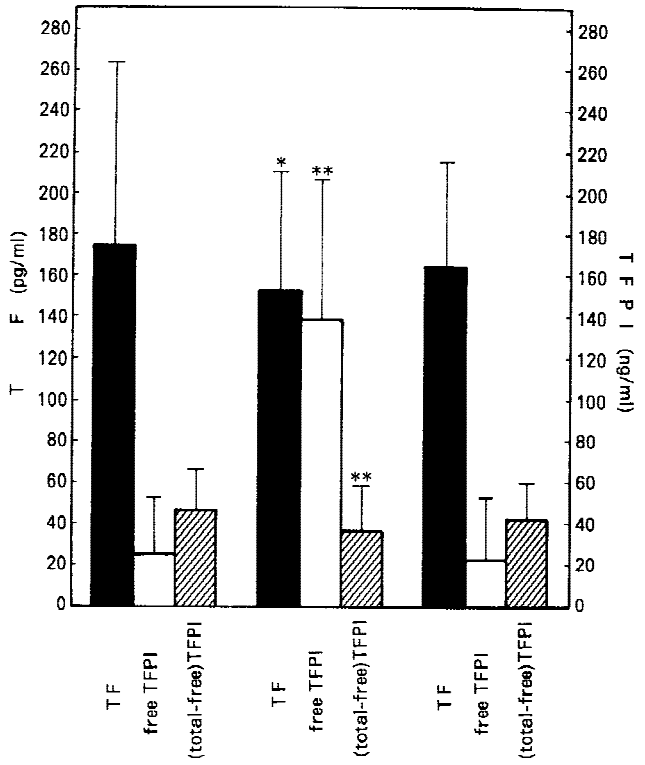

Fig. 5. Plasma levels of TF, free-TFPI, and (total-free) TFPI in patients with chronic coronary artery disease during PTCA. **P* < 0.05, ***P* < 0.01.

PTCA. The activity of plasma antithrombin and protein C significantly decreased in patients with coronary disease 1 hour after PTCA ($P < 0.01$), but they returned to normal range 1 day after PTCA (Fig. 1 and 2). Plasma protein C antigen level also decreased 1 hr after PTCA. There was no significant difference in the plasma levels of APC-PCI complex before (10.7 ± 6.1 ng/ml) and 1 hr after PTCA (10.8 ± 6.4 ng/ml) (Fig. 3). Plasma levels of D-dimer, TM and *PAP* significantly decreased 1 hr ($P < 0.05$), and the plasma level of PPIC 1 day after PTCA ($P < 0.01$) (Fig 4). One hour after PTCA, the plasma levels of TF and (total-free) TFPI significantly decreased ($P < 0.05$), but the plasma levels of total- and free-TFPI significantly increased ($P < 0.01$). These changes returned to normal range 1 day after PTCA (Fig. 5).

DISCUSSION

Hemostatic abnormalities have been previously reported in patients with AMI [7,21]. Plasma levels of TAT, *PAP*, D-dimer, and APC-PCI complex are significantly increased in patients with AMI [21], indicating that they are in hypercoagulable state. In our present study, patients with OMI and EAP had increased levels of D-dimer, SFM, and PPIC, suggesting that patients with chronic coronary artery disease have also hypercoagulability, although not severe. Patients with OMI and EAP showed increased plasma levels of TF, but normal plasma levels of total-TFPI, free-TFPI, and (total-free) TFPI. Plasma levels of both total- and free-TFPI are elevated in many patients with AMI [22]. Increased plasma level of TFPI was reported in patients with DIC [23] and severe sepsis [24], but not in those with deep vein thrombosis or pulmonary embolism [22]. Our findings suggest that the increase in the plasma level of TFPI may result from the release of TFPI from severe ischemic tissue or necrotic tissue.

One hour after PTCA, APTT, and *PT* were significantly prolonged and the plasma levels of total- and free-TFPI were significantly increased. Plasma TFPI may be released from vascular endothelial cells after injection of heparin [25]. Plasma levels of TF and (total-free) TFPI significantly decreased 1 hr after PTCA. These findings suggest that TFPI from vascular endothelial cells might be consumed during the PTCA procedure. The plasma activity of antithrombin and protein C significantly decreased 1 hr after PTCA, but it returned to normal range 1 day after PTCA. The fact that the plasma antigen level of protein C also decreased 1 hr after PTCA, suggests that these inhibitors were consumed during PTCA. The

deficiency of protein C or antithrombin is well recognized as a risk of thrombotic disease [26,27]. However, there was no significant change in the plasma level of APC-PCI complex before PTCA and 1 hr after PTCA, indicating that antithrombin and protein C were consumed by activation of the coagulation system. Plasma levels of D-dimer, SFM, TM and PPIC significantly decreased 1 hr after PTCA, and the plasma level of *PAP* 1 day after PTCA. These data suggest that the hypercoagulable state transiently improved after PTCA, and that the decrese of fibrinolisis continues up to 1 day after PTCA. The decrease of fibrinolysis in patients with AMI was reported to be due to increased plasminogen activator inhibitor I levels [28].

In conclusion, TFPI, protein C, and antithrombin transiently decreased during PTCA, and fibrinolytic activity also decreased 1 day after PTCA. These abnormalities may cause restenosis of coronary artery.

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